FOSTERING COMMUNICATION AND COLLABORATION

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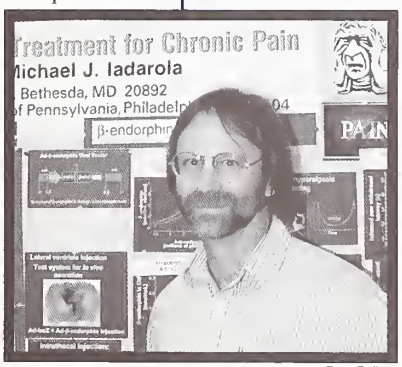
PAIN, CNS DISORDERS TARGET OF NOVEL GENE THERAPY PATHWAY

by Fran Pollner

NIDCR researchers have pioneered a new pathway to ameliorate chronic pain: gene transfer by recombinant adenoviral vector to deliver a β-endorphin gene directly into the meningeal tissues surrounding the spinal cord.

After long trial and error (see "Commentary," p. 11), they came up with a magic vector-target combo that induces nonneuronal cells to bathe the cord in analgesic balm.

The minigene is constructed so that the connective tissue cells of the pia mater will secrete β-endorphin into the cord and the cerebrospinal fluid (CSF). The strategy has relevance for neurodegenerative disorders and spinal cord injuries as well. Part of the Clinical Center's new bench-to-bedside initiative, the research was done in collaboration with the University of Pennsylvania in Philadelphia.



Fran Pollner

Michael Iadarola tells his research story—see page 11.

"We are totally pumped up. This approach is working, at least in the animal model," said Michael Iadarola, explaining his poster on "Viral Gene Transfer Approaches to Treatment for Chronic Pain" at the NIH Research Festival. Delivery of the β-endorphin gene into the CSF, he said, resulted in the rat's failure to exhibit the typical reaction to pain that would have resulted from an inflamed paw.

The brain or spinal cord tissue is not as hospitable a destination as the CSF space, said Iadarola, chief of the

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TWO MORE-THAN-MODEST PROPOSALS: AN NIH ACADEMY AND A GRADUATE SCHOOL

by Fran Pollner

Plato concluded that knowledge meant searching for truths that are independent of the observer and could be taught to others. He acted on this . . . belief by founding the Academy, a shady gathering spot just outside the walls of Athens. . . . The Academy became so famous as a gathering place for intellectuals that it continued to operate for 900 years after Plato's death. . . .

—a Plato scholar, on the Internet

It worked for Plato, and it lasted 900 years. Now those who envision greater and lasting diversity in the biomedical research workforce are proposing an Academy for NIH. Unlike Plato's singular Academy in ancient Athens, however, the NIH Academy would not stand alone in modern Bethesda but would have counterpart sites throughout the extramural research community.

At least that's the idea of a committee formed to brainstorm strategies to enlarge the variety of faces of those who conduct research at the bench and the bedside. Indeed, a primary recommendation in the newly released *Report and Recommendations of the Committee for Recruitment of a Diverse Workforce in Medical Research* (or, more popularly, the Slavkin report, after committee chairman Harold Slavkin, NIDCR director) is "creating The NIH Academy."

In this issue of *The NIH Catalyst*, Deputy Director for Intramural Research Michael Gottesman elaborates on the concept and structure of the proposed Academy (see "Toward an NIH Academy," page 2). He previewed the major points of the Slavkin report at the semiannual meeting in December of the Advisory Committee to the Director of NIH (ACD)—and made the case also for an NIH graduate school, which, if it were to be established, would be but one part of the NIH Academy. The doctoral degree it would confer would reflect the patient orientation of the training here.

At the ACD meeting, there was nary a contentious word about the NIH Academy from any of the distinguished panelists who constitute the NIH director's outside advisory group. They were less uniformly sanguine over the prospect of an NIH graduate school.

Academy: Yes

The NIH Academy would enhance and choreograph what are now largely disconnected programs at NIH geared to the range of students from high school through the postdoctoral years. The programs are designed not only to intensify an existing fascination with biomedical research but also to open the field up to those for whom it might not otherwise be accessible.

In coordinating these programs within a formal structure, the NIH Academy

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Toward an NIH Academy

Michael Gottesman

In addition to its pre-eminent role as a research institution, the NIH has a formidable reputation for the quality of its trainees. Contributing to the sense of excitement and scholarship on campus are numerous students at all levels of education—high school, college, postbaccalaureate, graduate, and postdoctoral. We have a responsibility to these students to provide the best quality training and mentoring that we can. To this end, our ethics and conduct committee, under the direction of Joan Schwartz, has completed a new *Guide to Training and Mentoring in the Intramural Research Program* (see pages 4–5), which has recently been distributed to all trainees and principal investigators at NIH. I hope you have had an opportunity to read this pamphlet and discuss it with your trainees, colleagues, mentors, and supervisors.

This *Guide* is just the beginning. Over the past few years we have become aware that there are other important ways in which we can improve training opportunities at NIH. The recent Slavkin committee's *Report and Recommendations of the Committee for Recruitment of a Diverse Workforce in Medical Research* points out that an important way to help guarantee more attention to research problems related to health disparities is to train a cadre of scientists who themselves come from disadvantaged backgrounds.

We now need to do much more—working both harder and smarter—to attract a diverse group of scientists-in-training and to provide an intellectually challenging yet nurturing environment.

It has been apparent for many years that the pool of researchers from which NIH chooses its junior and senior faculty has far fewer African-Americans, Native Americans, and Hispanic Americans than found in the general U.S. population, and we have created a conglomeration of programs to address this problem. We now need to do much more—working both harder and smarter—to attract a diverse group of scientists-in-training to NIH and to provide an intellectually challenging yet nurturing environment.

The Slavkin report strongly recommends that the NIH develop a training Academy whose goal is to identify, recruit, and nurture talented young scientists from all over the country. Major components of the Academy include an active recruitment program, better coordination of existing programs, more uniformly high quality mentoring and training experiences, and continuity of programs from high school through college and graduate school, as well as postdoctoral experiences (both at NIH and among the NIH and other extramural academic institutions) and the possibility of housing on or near the NIH campus. Among the various Institute programs for recruiting young investigators, many of these components already exist. The NIH Academy would give clearer definition and cohesion to these programs, would increase the visibility of NIH as an important training institution, and would guarantee more uniformity in quality among the various programs at NIH.

In addition to the Institute summer programs, which most NIH scientists know about, there are several programs supported by the Office of the Director that illustrate some of the approaches that might be taken by the new NIH Academy. With joint sponsorship from the Howard Hughes Medical Institute and the FAES, NIH has run a highly selective program for local high school students for the past 12 years. In addition to a laboratory experience, this program includes a weekly session at which students present their own research and learn about the research of their colleagues. The Undergraduate Scholarship Program, under the direction of Marc Horowitz in the Office of Loan Repayment and Scholarship, is in its third successful year of recruiting disadvantaged students from around the country. These students work at NIH in the summer, are housed together and carefully mentored, and have college tuition and expenses paid by NIH. For each year of NIH support, they owe a year of service to NIH. This program has already been the source of many outstanding NIH students, and we look forward to their return as fellows and investigators at NIH.

Our two medical student programs are also quite successful. The Howard Hughes Medical Institute–NIH Scholars program brings second-year medical students to NIH for a research lab experience, and the Clinical Research Training Program brings third-year medical students here for a clinical research experience. Each of these programs provides housing, tutorials, mentorship, and the full range of research opportunities available at the NIH. Many of the students serve as role models and mentors for other students on the campus, demonstrating how cross-age mentoring can be a very positive tool in the training of students at all levels.

I will be appointing a working group of interested NIH scientists, educators, and administrators who will make specific recommendations for creating a more inclusive training Academy at NIH. I welcome your ideas, and we will be depending on you for support as we implement the recommendations of this working group.

—Michael Gottesman
Deputy Director for Intramural Research

Just Ask!

Dear Just Ask:

Do you know if there are mail groups for researchers by country? I think I recall seeing a few country interest groups (e.g., Italian, Japanese, Chinese) advertised on the DDIRBB. Recently, we had an inquiry from a sponsor who wants to know if there is an Italian e-mail group (or one for any other country). Our branch does not maintain such mail groups, but wondered if you knew about their existence. I would be grateful for any information you might have. Thanks in advance.

—Valerie Katsouros
Fogarty International Center

Celia Hooper

Susan Chacko

Dear Valerie:

There are mailing lists related to almost every discipline, interest, country, religion, age group, and society; the only problem is to find the one that you're looking for!

An NIH researcher might be most interested in a local mailing list, which might have announcements of local resources and meetings.

A good place to start, then, is the NIH LISTSERV. LISTSERV is a software package that manages e-mail lists, and the NIH LISTSERV manages all the mailing lists on campus.

Point your web browser to <**http://list.nih.gov**> and click on the 'Browse' button, which pulls up a long list of all the mailing lists that our LISTSERV manages. There's a convenient 'Search' box on top, so let's search for 'Italy.' We should probably try 'Italian' as well, since this list might be called 'ITALIAN-L,' or describe itself as 'A list for Italian researchers.' In this case, neither word was found.

Random searches for other country names showed that there is a Chinese Scholar Association List (CSANIH-L), an NIH-FDA Chinese American Association list (NIH-FDA-CAA), the Hispanic Employee Organization (HEO-INFO-L), Greek scientists at NIH (GREEKS), Japanese scientists at NIH (JAPAN-L), and International Activities at NIH (INTERNATIONAL-L), among others. Clicking on each name lets you join ('subscribe' to) or leave ('unsubscribe'). If you've already subscribed to this list, you may be able to browse the list archives.

Before I'd subscribe to a list, I'd probably want to find out more about it. So I sent an e-mail message to <Listserv@list.nih.gov> saying 'Review JAPAN-L.' The message that came back indicated that anyone can subscribe to this list, but that most messages posted on the list are written in Japanese, which would not suit someone who does not read the language.

No Italian list has turned up, so it's time to look further afield. The main LISTSERV page has a link to 'Mailing List Search Sites,' which features four gigantic catalogs of mailing lists around the world—CataList, Publicly Accessible Mailing Lists (PAML), Liszt, and Tile.net. I searched for 'Italy Italian' in each of them, which should return lists with either 'Italy' or 'Italian' in their name or description. CataList (<**http://www.lsoft.com/catalist.html**>) gave me COMUNES_OF_ITALY, about Italian geneology and culture. PAML (<**http://www.neosoft.com/cgi-bin/paml_search/**>) returned 23 different lists, including yoga, an Italian-language list about yoga. (See what I mean about mailing lists on every possible topic?) Liszt (<**http://www.liszt.com/**>) returned 115 lists that dealt with everything from an Italian-language guitar list to a list about soccer in Italy! Tile.net (<**http://tile.net/listserv/**>) gave me 33 lists.

As you see, it's worth searching all four sites, since they compile their information by different methods and thus give different results. If you're searching for several words (such as 'Italy' OR 'Italian'), note that some search sites will let you specify 'All words' or 'Any words' for your search.

Most of these search sites will let you click onto a list to get some minimal information about it, but it's worth sending the 'review LISTNAME' command to the subscribing address to get a fuller description.

Chances are that you will find an interesting list by trying these tips. If you don't find an appropriate list, and if you think there is sufficient interest in your own favorite topic, you could always start a mailing list yourself (See "Call for Hispanic Scientists" below.) The application form can be filled out online at the NIH LISTSERV web site, and the list will be set up in one business day. Check out the NIH LISTSERV web site for more information.

—Susan Chacko

URL Windows on the World

NIH LISTSERV: <**http://list.nih.gov**>
CataList:<**http://www.lsoft.com/catalist.html**>
PAML: <**http://www.neosoft.com/cgi-bin/paml_search/**>
Liszt <**http://www.liszt.com/**>
Tile.net: <**http://tile.net/listserv/**>

Attention: All Hispanic Scientists

The NIH Fellows Committee, with the support of the Office of Education, is organizing a Hispanic Scientists Directory. This directory is meant to include all intramural (NIH and FDA-CBER) Hispanic scientific personnel (basic research and clinical postdoctoral fellows, staff scientists and clinicians, nurses, technicians, predoctoral students, tenure-track and tenured investigators).

This directory should help identify all Hispanic scientists at NIH/FDA-CBER and their scientific contribution to the NIH scientific community. It should also encourage the interaction and exchange of ideas and information among Hispanic researchers. If you would like to be included in this directory, please send your name, e-mail address, and phone and fax numbers to

<**vazquez@cber.fda.gov**>

or contact Nancy Vázquez at (301) 827-1774. ■

Mentoring at NIH: New Guide Released As Fellows evaluate Current Practices

by Joan P. Schwartz, Ph.D., NINDS Assistant Director, OIR

Mentoring has become a hot topic in the academic world—and it has had its fair share of attention in the NIH intramural research program. Three years ago NIAID's Richard Asofsky and I coauthored a column in *The NIH Catalyst* entitled "Training of Postdoctoral Fellows: A Shared Responsibility" (March-April 1996, p. 6). In 1997, Michael Gottesman, DDIR, expounded on the subject in a talk to NIH fellows and then developed that talk into a *Catalyst* column (November-December 1997, page 2) that outlined his picks for the key elements of postdoctoral training at NIH (see below). And now, at the DDIR's request, hot off the presses comes *A Guide to Training and Mentoring in the Intramural Research Program at NIH,* written by the NIH Committee on Scientific Conduct and Ethics. The *Guide* is especially timely because the Fellows Committee has also conducted a mentoring survey of NIH postdoctoral fellows. A preliminary scan of responses suggests that the fellows do not find NIH investigators to be uniformly excellent at mentoring (see "Mixed Reactions").

Guide-lines for Mentoring

There are three sides to the mentoring story or, rather, three sets of responsibilities—those of the mentor, those of the trainee, and those of the institution. All are addressed in *A Guide to Training and Mentoring*. Not only will fellows appreciate the *Guide*'s roundup of reasonable expectations of an NIH training program, but its review of mentoring responsibilities should prove useful for supervisors as well. The *Guide* defines a mentor as a "person who has achieved career success and counsels and guides another for the purpose of helping him or her to achieve like success."

Fran Pollner

Joan P. Schwartz

All investigators at NIH should be mentors to their fellows, although many fellows will identify additional mentors both at NIH and elsewhere. That 25 percent of fellows responding to the mentoring survey reported that they had no mentor should give us pause.

In what areas should mentoring be provided? First and foremost is scientific investigation, because all scientists come here to carry out research of some type. Thus, teaching a fellow how to choose a project, to ask important scientific questions, and to design experiments and carry them out is essential—and that's one area where most fellows report they are receiving adequate to outstanding advice and support.

Regular lab meetings and talks with supervisors are also key to postdoctoral fellows' development as scientists; yet 20 percent of fellows reported interacting less than once a month with their supervisor, either individually or in a lab meeting.

Other elements of an effective training experience include opportunities to attend seminars and meetings and to review the relevant scientific literature; opportunities to present one's work informally at lab seminars, and more formally at NIH or at meetings, in order to develop those oral skills that are essential regardless of the type of job ultimately chosen; and opportunities to prepare the first draft of manuscripts detailing one's research and to master the art of writing scientific papers—which may take longer than a supervisor might like, but which is an absolutely essential skill for every fellow on campus to acquire.

Give and Take

Less obvious skills fellows need to learn—but are seldom taught—include the ability to negotiate and hone their diplomatic capabilities, a topic covered in a previous *Catalyst* column (March-April 1998, p. 6). Indeed, such skills are so useful and so overlooked that our new NIH Ombudsman, Howard Gadlin, might do well to put together some NIH-wide seminars on the subject.

Integral to all such interactions is the ability to communicate with others, and not only about the science in the lab. A major theme in the fellows' responses to the mentoring survey was the need for evaluation, of both the mentor and

What Every Trainee Should Learn at NIH

- Doing Science
- Choosing A career path
- Reading The Literature
- Networking
- Communicating
- Respecting Resources
- Conducting Ethical Science
- Mentoring
- Forming Collaborations
- Negotiating

—Michael Gottesman, DDIR "Training Scientists at NIH" The NIH Catalyst, *Nov.–Dec. 1997*

Mixed Reactions to Mentoring in Fellows' Survey

In the summer of 1998, the NIH Fellows Committee conducted a web-based survey of postdoctoral fellows at NIH to assess the mentoring experiences of NIH fellows. The survey questionnaire, developed in consultation with outside experts, covered demographic characteristics, supervisor-fellow interactions, and promotion of professional development. Several strategies were used to inform the more than 2,300 postdoctoral fellows about the survey, including mailed and e-mailed flyers and notices posted on the Fellows' web site. Twenty percent of the postdoctoral population—465 fellows—responded anonymously to the survey. Some preliminary findings are presented here.

IRTAs accounted for 45 percent of respondents, and visiting fellows for 31 percent; the remainder were research or clinical fellows or on other fellowships. Half of the survey respondents had been at NIH for one to two years and 9 percent for less than one year. Slightly more than half of the respondents were men. A comparison of respondents to the broader NIH fellows population suggested that women were more likely to respond than men and that fellows who had been at NIH longer were more likely to respond.

More than two-thirds of respondents reported that they had a mentor and that that individual was their supervisor, but one-quarter reported that they had no mentor. More than half of responding fellows reported meeting individually with their supervisors at least weekly, but 20 percent reported meeting with them less than once a month. Access to their supervisor was reported to be adequate by more than three-quarters of the survey participants. Advice from a supervisor was more likely to be described as adequate when the project was going well than when it was stalled (78 percent v. 59 percent). About half of respondents said they were accomplishing their goals for their fellowship training, but 29 percent said they were not. Most of the fellows (81 percent) felt that their supervisor's expectations of them were reasonable.

The Fellows Committee anticipates that the full survey results will aid the NIH community in its continuing efforts to provide the best possible postdoctoral training for future scientific leaders. A final report will be forthcoming. ■

the fellow. Many respondents suggested that it would be wise to have to sit down once a year with one's supervisor and evaluate how each is doing in the relationship; most fellows expressed an interest in getting a yearly "progress report" on their research. This is perhaps the toughest type of communication we as scientists have to do, but it is also essential. The *Guide* suggests yearly evaluations of each fellow, including assessments of both research progress and career plans.

Beyond NIH

Career planning is a major concern of fellows and one where many feel that support from supervisors is minimal. As the possibilities for different types of careers expand, and careers involving more collaborative work rather than independent positions become more prominent, the supervisor or mentor often requires as much education as the fellow.

Those of us who grew up in a system where it was assumed that everyone would end up in academia need to recognize the diversity of career options now available and encourage our fellows to do likewise. NIH, and in particular the NIH Fellows Committee, offers an array of talks and workshops on new career opportunities for the 21st century; investigators should be aware of these and actively encourage their fellows to participate. Investigators should also network on behalf of their fellows by learning from colleagues about career opportunities and consciously promoting the visibility of their fellows' research—and they should enable their fellows to meet their colleagues so they can start establishing their own networks.

The final area of training essential to all scientists involves the responsible conduct of science. Here the role of supervisor-mentor is critical because much of this training comes directly from the examples of ethical, and unethical, actions fellows observe in their labs. Discussion of the standards contained in the *Guidelines for the Conduct of Research in the Intramural Research Program at NIH* in the laboratory setting can ensure that everyone agrees on what these standards are. Supervisors should also foster a sense of responsibility in their fellows for the appropriate use of public resources and direct them to the necessary courses on human subjects research, care and use of animals, and laboratory safety issues.

Train the Trainers?

The bottom line is that NIH investigators need to be mentors to their fellows—in many areas—and NIH fellows, according to the mentoring survey, are not completely satisfied with the mentoring they are receiving. Research from the University of California at Irvine suggests that "training is . . . important to the success of mentoring." Will reading *A Guide to Training and Mentoring in the Intramural Research Program at NIH* be sufficient? Should each lab group discuss the ideas in the *Guide* as a group and agree on how to implement some of them? Should PIs be offered courses that might improve mentoring skills? As NIHers throughout our campuses read the *Guide* over the next few months, we hope they will tackle these questions.

Postdoctoral fellows are not only a valuable resource for the labs right now; they are the scientific leaders of the future. The skills NIH postdocs develop during their time here are the foundation for their success in the future and a measure of the success of NIH and its scientist-mentors. ■

New Training Proposals

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would fulfill the Slavkin recommendation that it "serve as a nexus for recruiting and training a diverse population of students to pursue careers in the biomedical sciences," Gottesman said.

Summarizing the "critical elements" for the Academy's success, Gottesman mentioned "intensive mentoring, personal attention in the lab, and, even more important, a residential facility" that would provide housing and meeting space for both educational and social activities, enabling "vertical mentoring" by more advanced students. The aim, he said, is "continuity" of experience from high school through college and into and beyond the graduate and postdoctoral years. Moreover, the programs implemented at NIH would also be disseminated to extramural sites—to truly change the complexion of the biomedical workforce across the country.

A salient feature of the Academy would be experiencing "real-world" problems in local communities, an important departure from the ivory-tower academic setting that would enable students to see the effect of their training in the real world. ACD members expressed particular satisfaction with this aspect of the Academy.

Graduate School: Maybe

Several, however, questioned the wisdom of the Academy's including a formal degree-granting graduate school, an idea that has support among NIH leadership. The goals of an NIH graduate program, Gottesman said, would be to fill in those areas in which the country's graduate education is flagging, to provide much-needed doctoral-level training for M.D.s and others in new and highly specialized areas of clinical and translational research. A formal graduate program within the Academy, he observed, would also "certainly enhance diversity during the very years when diversity tends to drop off."

ACD member Shirley Tilghman, professor of molecular biology at Princeton (N.J.) University, disputed the notion that there is a "national need for a new graduate school. There's a need for new kinds of training, and that should be done at the postdoctoral, not the Ph.D., level," she contended. (Tilghman was chair of a National Research Council committee that last year concluded that there are more Ph.D.s in the life sciences in the United States than the U.S. job market can happily accommodate. The Tilghman report urged that "there be no further expansion in the size of existing graduate-education programs in the life sciences and no development of new programs, except under rare and special circumstances, such as a program to serve an emerging field or to encourage the education of members of underrepresented minority groups.")

Gottesman countered that one year of patient-oriented training would be "totally insufficient" in a field that is growing so rapidly, especially with new information pouring out of such efforts as the Human Genome Project. Moreover, he said, Ph.D.s with an interest in clinical research would be "greeted with delight" anywhere in the country.

ACD member Eric Kandel, professor of neurobiology and behavior at Columbia University in New York, suggested that an NIH graduate program in clinical investigation could serve as a national model. "Everyone acknowledges there is a crisis in clinical investigation, and for the first time, people are seeing clinical problems as relevant to basic research. NIH is uniquely positioned to provide leadership," Kandel said.

Eric Lander, professor of biology at the Massachusetts Institute of Technology in Cambridge, Mass., said that there is "no excuse for an NIH grad school unless it's distinctive."

That distinctiveness, said Marc Kirschner, professor and chair of cell biology at Harvard Medical School, Boston, could be demonstrated in a curriculum that featured such courses as pathophysiology, bioinformatics, and clinical problems.

To initiate a graduate program, Gottesman told the ACD, "we need your advice and local university input"—as well as "legislation enabling NIH to be a degree granter, state accreditation, a graduate dean, staff, and curriculum."

The NIH graduate school will be on the agenda at the next ACD meeting in June, at which time, Gottesman said later, a proposal will be presented to the panel. Meanwhile, consultations with outside experts are proceeding and an NIH-wide "town meeting" of people interested in contributing to a graduate program will be held in April or May. Date, time, and location will be forthcoming, Gottesman said. ■

Seeking Diversity in the Scientific Ranks

Celia Hooper

Joseph Curtis

Celia Hooper

NICHD's Hamid Khan and Dorothy McKelvin

Bill Branson

Arlyn Garcia-Perez

During a panel discussion February 11 in Lipsett Auditorium on the importance of diversity in the biomedical community, Joseph Curtis (left), a former postdoc in the lab of NCI's Ira Pastan, said that African-Americans are "even less well represented outside academia" than within it and therefore have a "chance to make a huge impression." Now a Maryland-based independent consultant to small companies seeking FDA approval for diagnostics, Curtis said he is often the "first African-American [his clients in the international biotechnology community] have ever seen." NICHD's Hamid Khan (center, left) cited two myths that block acceptance of some women and minority scientists: that they are too "laid back" or nonproductive and that they "can't communicate" because they have accents or speak softly. NHLBI senior investigator, Arlyn Garcia-Perez (right), who insisted she has "never spoken softly," told the audience that "NIH is perceived as unfriendly" to minority scientists. Making the community more diverse, she said, would strengthen it. Garcia-Perez joined the Office of Intramural Research, OD, this year as assistant director.—C.H.

Increased Enrollment Anticipated for Second Year Of Duke-NIH Master's Program in Clinical Research

What makes a good clinical researcher? What basic tools do researchers need to translate what seems promising at the bench into a new therapy at the bedside? Research design, statistical and decision analysis, research ethics, project management—what role does each play in the conduct of a solid clinical trial?

Clinical fellows and other health professionals at NIH have a unique venue for exploring answers to those questions, thanks to a cooperative training program between the NIH Clinical Center and the Duke University School of Medicine in Durham, N.C. The training culminates in a Master of Health Sciences in Clinical Research conferred by Duke.

"In the past, techniques of clinical research were passed from a seasoned mentor to a willing student. In today's research arena, that's not enough," observes John Gallin, Clinical Center director and a prime mover of the Duke-NIH collaboration. The clinical researcher, he says, needs a thorough grounding in the clinical research process.

Duke University initiated its Master of Health Sciences in Clinical Research program more than a dozen years ago to provide that grounding and expertise. The collaboration with NIH marks the university's first efforts at making the program more widely available. Students here at NIH attend classes by way of videoconferencing.

Other classes are taught onsite by adjunct faculty, such as Ezekiel Emanuel, chief of the CC's Department of Clinical Bioethics, who teaches "Ethical and Regulatory Aspects of Human Subjects Research." For the upcoming academic year, Emanuel will be joined by Art Atkinson, who will offer an elective in "Principles of Clinical Pharmacology."

The program's first class of 14 students was admitted last September. This spring, four of the students will complete the required course work for the program. Gallin anticipates an expanded class in the 1999-2000 academic year and welcomes applications from both intramural and extramural divisions.

The degree program requires 24 units of graded work plus a research and thesis project, which carries 12 units of credit. "The program's design," Gallin says, "encourages the meshing of clinical and academic training. It can be completed in two 16-week sessions, although degree-seekers typically spread the course work over two years."

Applications are currently being accepted for the 1999–2000 academic year and are available in the NIH Office of Education, Building 10, Room 1C129.

Bill Branson

Seated (left to right): Marjorie Garvey, Raphael Schiffmann, Joshua Kouri; standing (left to right): Gabor Illei, Joseph Hoxworth, Richard Messman, Douglas Shaffer, Kara Sovik, Irini Sereti, Susanne Goldstein. (Not pictured: Salman Azhar, Richard Nahin, Jorge Tavel, George Wittenberg)

All participants must be formally admitted to the training program by the Duke University School of Medicine. The deadline for receipt of applications is **April 15, 1999**. Applicants who have been accepted into the program will be notified by July 1, 1999. Questions about the program may be directed to William Wilkinson, program director, at **<tpcr@mc.duke.edu>**.

For more information regarding course work and tuition costs for the 1999–2000 academic year, visit the program's web site at **<http://www.cc.nih.gov/ccc/cc_duke/info.html>**.

FARE 2000: More NIH Fellows to Reap Awards in Millennial Competition

The sixth annual NIH-wide Fellows Award for Research Excellence (FARE) 2000 competition will again provide recognition for the outstanding scientific research performed by intramural postdoctoral fellows. Winners of FARE awards will each receive a $1000 stipend to use for presenting their work at a meeting in the United States. Fellows who apply to FARE submit an abstract of their research; abstracts are then peer reviewed in a blinded study section. The award must be used between October 1, 1999, and September 30, 2000. The FARE 2000 competition is open to postdoctoral IRTAs, visiting fellows, and other fellows with less than 5 years total postdoctoral experience in the NIH intramural research program. Pre-IRTAs currently enrolled in a Ph.D. program may also compete. Visiting scientists and fellows must not have been tenured at their home institution.

Questions about eligibility should be addressed to your institute's scientific director. Fellows are asked to submit their application and abstract with an online application available from **<ftp://helix.nih.gov/felcom/index.html>**. Applications will be accepted from **May 3–June 1, 1999** (12:00 noon, E.S.T.). Winners will be announced by September 1999. Questions about FARE 2000 should be directed to **<FARE2000@box-f.nih.gov>** or to your institute's Fellows Committee representative. Information is also available at the Fellows Committee site at **<ftp://helix.nih.gov/felcom/index.html>**. FARE 2000 is sponsored by the NIH Fellows Committee, the scientific directors, the NIH Office of Research on Women's Health, and the NIH Office of Education. The FARE 2000 award is funded by the scientific directors and the NIH Office of Research on Women's Health.

Last year's FARE 1999 was very successful; 666 abstracts were submitted, and 130, or 19.5 percent, were funded. The FARE 2000 competition will provide an even higher level of funding—25 percent of applicants will receive a $1000 award. Through the month of June, sets of related winning FARE 1999 posters are being displayed outside the Visitor's Information Center in Building 10 on Wednesday afternoons, in conjunction with the Wednesday Afternoon Lecture series. ■

Vaccine Pursuit Accelerated In NIAID Malaria Research program

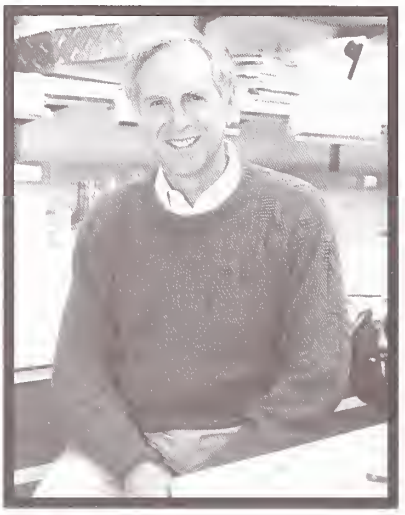
by Doug Loftus

The quest for a malaria vaccine is expanding its clinical dimension in the form of a new initiative headed by Louis Miller, chief of NIAID's Laboratory of Parasitic Diseases (LPD), the home of NIH's core program of malaria research. Now added to the LPD research agenda—which includes the parasite-host interaction, the genetics of pathogenesis and drug resistance, and immunological strategies for prevention and intervention—is the highly specific task of producing a blood-stage vaccine that would elicit an immune response—primarily antibodies—capable of intervening at the critical disease-producing phase in the life cycle of the parasite *Plasmodium falciparum* (see sidebar "Mosquitoes, Parasites, and Malaria").

Vaccine Development

The vaccine, says Miller, who is also chief of the Malaria Cell Biology Section, would target the parasite proteins that mediate merozoite entry into red blood cells (RBCs) and the binding of RBCs to endothelium (the endothelial-RBC interaction prevents RBC transit through the spleen and subsequent destruction, providing a "safe haven" for merozoite proliferation).

In close collaboration with Stephanie James, deputy director of NIAID's extramural Division of Microbiology and Infectious Diseases, Miller will oversee the activities of a research program designed, he says, to operate "like a small company." He calls it "a major undertaking," pointing out the myriad logistical hurdles involved in making products that will be used in people. Expression systems, production methods, formulation, and testing must each be carefully evaluated and controlled, with a relentless attention to procedural details.



Douglas Seeley

Louis Miller

Miller believes that NIH's unique brand of long-term, committed support has brought the intramural malaria program to where it is today, poised to advance to the next round of discovery and development. He admits, however, that stepping into the clinical realm was not his natural inclination. "I would have never considered it," he says, but did so at the urging of David Kaslow, who has been chief of the Malaria Vaccines Section (see *The NIH Catalyst*, November-December 1997, page 1). "It was a turning point in the lab," Miller says, noting that Kaslow "single-handedly ran against the grain" in advocating a bench-to-clinical approach. "Unfortunately," he adds, "David is leaving so I've taken over the group. We're trying to recruit people to fill in all the different aspects of vaccine development."

Although the vaccine program is just beginning to take shape, portions of the molecular strategy are already being developed. Miller believes that, given the number of variables associated with producing an effective clinical-grade reagent, initial efforts should be tightly focused on a single target molecule. They will start with the merozoite surface protein MSP-1 because high antibody titers against MSP-1 have been correlated with protective immunity in animal studies, and among Africans, resistance to disease also is correlated with serum antibodies against parasite surface proteins. For help with handling the scientific details of vaccine design and development, Miller feels fortunate to have an advisory panel of colleagues from NIAID's Laboratory of Infectious Diseases, whose experience in developing vaccines for rotavirus (see "More than Two Decades of Research Culminates in Rotavirus Vaccine," p. 9) and other viruses is a valuable asset.

With all the pieces about to fall in place, Miller acknowledges that the development path will be long and challenging. He envisions taking a product through Phase II clinical trials—which could take "5–15 years"—and then handing it to industry for final testing

Mosquitoes, Parasites, and Malaria

According to the World Health Organization, the female *Anopheles* mosquito transmits malaria to about 500 million people a year in more than 90 countries of Africa, Asia, and South America. The parasite *Plasmodium falciparum* causes the most severe form of malaria in humans, leading to the death each year of about two million people, mostly children.

Anopheline mosquitoes inject *Plasmodium* sporozoites into the human bloodstream while feeding. These sporozoites find their way to the liver, where they mature into merozoites and again are released into the blood. Merozoites infect red blood cells (RBCs), where they obtain the hemoglobin they need to proliferate. The RBC eventually bursts, releasing more merozoites into circulation; these cycles of infection and cell rupture produce disease. Occasionally, a merozoite within a red cell switches to a sexual form called the gametocyte. A feeding mosquito can take up RBCs harboring gametocytes, which further develop in the mosquito gut and eventually give rise to sporozoites that infect the mosquito salivary gland, ready to be injected into the next human host.

Discoveries near the end of the last century led to a better understanding of malaria's cause and mode of transmission. The use of pesticides and the development of synthetic derivatives of quinine, a component of cinchona bark long known for its antimalarial properties, were instrumental in bringing malaria under control in the first half of this century. However, the effectiveness of these methods has waned progressively over the past 30–40 years, resulting in a resurgence of malaria as a major public health concern in many parts of the world. ■

phases and bringing it to market. According to Miller, even the ultimate consumers of the anticipated vaccine recognize the need for persistence and patience. "They're pretty sophisticated," he said, recounting discussions he'd had with village elders during a visit to Mali.

Drug Resistance

Proceeding apace in the LPD is research aimed at understanding the mechanisms of parasite resistance to drugs that, at least for a time, had triumphed over malaria. Drug resistance is now widely acknowledged as a critical problem in treating and reducing the spread of the disease. Thomas Wellems, chief of the LPD Malaria Genetics Section, observes that chloroquine, an inexpensive, easily administered first-line antimalarial agent, had been a boon to the public health comparable to penicillin until the emergence of chloroquine-resistant strains of *P. falciparum* over the last 30 years or so.

Wellems and his group have been trying to identify genes responsible for chloroquine resistance, and he now believes they are narrowing in on a candidate, which could make efforts to overcome resistance through drug design feasible. Citing the apparent reluctance of big drug companies to invest effort in developing antimalaria therapeutics, he suggests that they simply haven't known "where to start." The lack of molecular targets and simple in vitro culture methods mitigates against the high-throughput methods of drug discovery typically used in industry, he explains. "What we're missing in *P. falciparum* is a model system—we don't have one," he says. He explains that mice, for instance, which aren't susceptible to *P. falciparum*, can be infected with another *Plasmodium* species, but disease takes a significantly different course from that in humans.

LPD clinical research efforts also extend overseas, particularly to Africa. LPD Assistant Chief Robert Gwadz heads a three-pronged collaborative initiative with researchers at the National School of Medicine in Mali that encompasses molecular epidemiology, natural protective factors against severe malaria, and candidate vaccine strategies. Wellems observes that when projects are based in malaria-endemic regions, the "most relevant questions" tend to surface, helping to focus research directions. ■

More than Two Decades of Research Culminates in Rotavirus Vaccine

by Fran Pollner

It was last summer that the clinical research that Albert Kapikian and colleagues embarked upon in 1974 culminated in a gift package to the world—a licensed live-virus oral vaccine against rotavirus, the most important known cause of severe diarrheal disease, a condition responsible for more years of life lost worldwide than any other, except for lower respiratory tract infections.

Head of the epidemiology section in NIAID's Laboratory of Infectious Diseases, Kapikian relived his decades-long research "adventure" in a plenary presentation during the NIH Research Festival last October. A month later, he received the 1998 Children's Vaccine Initiative Pasteur Award for Recent Contributions to Vaccine Development, an honor he shared with two other scientists—Ruth Bishop, who discovered the rotavirus at the Royal Children's Hospital in Melbourne, Australia, in 1973, and Roger Glass, whose work on rotaviruses began at NIH under Kapikian's direction and then moved to the Centers for Disease Control and Prevention, where he gathered epidemiological evidence that rotavirus infection is prevalent in developed as well as developing nations.

Fran Pollner

Albert Kapikian

"It's the single most important etiologic agent of severe diarrhea in infants and young children—everywhere in the world, including the United States, and everyone has had it by age 5," Kapikian said. "It's egalitarian; it has no regard for wealth or hygiene." Peak incidence is between 6 and 24 months in the United States and between 3 and 24 months in developing countries. The vaccine is designed to prevent rotavirus-induced severe diarrhea in children under 2—and the estimated 870,000 associated deaths a year, mostly in developing countries, as well as the large number of related hospitalizations and emergency room visits. It's not designed to protect against infection itself or mild diarrhea. "We're not concerned about reinfection when a child is older and the infection is less severe, but with that first infection with wild-type rotavirus, which is the severe one," he noted.

Collaborative clinical research with Washington's Children's Hospital from 1974 to 1982 established the presence of rotavirus in the stool of 34 percent of children admitted with gastroenteritis. That percentage typically rose to between about 60 and 70 percent in the coolest months, Kapikian recounted. Of 10 human rotavirus serotypes, four have been identified as "epidemiologically important," and it was against these four that vaccine efforts were targeted. "We sought to induce antibodies to the major outer-capsid protein, VP7, and eventually produced a quadrivalent rotavirus vaccine that incorporates VP7 specificity for each of the four epidemiologically significant strains," Kapikian said, summarizing years of his team's work. He added that "as in Jenner's 1796 smallpox vaccine, we used a live attenuated agent from a nonhuman host as an immunogen." They used a rhesus rotavirus strain to represent one of the serotypes and later generated rhesus rotavirus-human rotavirus reassortants for each of the other three serotypes to formulate the quadrivalent vaccine.

Between 1984 and 1997, "with tremendous collaboration throughout the U.S. and overseas," thousands of individuals were enrolled in 11 randomized, controlled trials, five of which—three in the United States, one in Finland, and one in Venezuela—were instrumental for FDA approval of the product license for RotaShield, issued to Wyeth-Ayerst, which had entered into a collaborative agreement with NIAID in 1987 (see "NIH Research Yields New Products," p. 12).

Asked about the cost of the vaccine, Kapikian said he'd discussed that issue with the company and that "tiered financing" would be used. "Profits made through sales in developed countries will be used to fund distribution elsewhere," he was assured, he said, noting also that the access issue is a "high priority of WHO." ■

The Fruits of Tech Transfer: NIH Research Yields Six New FDA Approvals in 1998

The NIH Office of Technology Transfer reports that six new products developed from research conducted by NIH scientists were approved by the FDA in 1998.

The six products emanate from five different institutes, and five of them are "firsts" of their kind.

Typically, there have been one or two such approvals yearly, says Steve Ferguson, OTT senior licensing specialist, who notes that the FDA action is the final step in actions initiated by the inventors, institutes, and OTT more than a decade ago, when the OTT was launched in response to changes in the technology transfer law.

"Our investments are starting to pay off now," he observes, and 1998, therefore, may mark the "beginning of an upward trend."

While all the approvals are gratifying, the RotaShield license (see below) is "particularly exciting," says Ferguson, because its history is longest (see "More than Two Decades of Research Culminates in Rotavirus Vaccine," p. 9). For more info, contact Ferguson at 496-7057, ext. 266, or at **<sf8h@nih.gov>**.

Synagis (Medimmune, Inc.)—a monoclonal antibody used for the prevention and treatment of serious lower respiratory tract disease by respiratory syncytial virus (RSV). RSV is the most common cause of pneumonia and bronchiolitis in infancy and early childhood. Synagis is the world's first monoclonal antibody licensed by the FDA for any infectious disease. (Nonexclusive Biological Materials License)
NIH Inventors: Robert Chanock, Brian Murphy, Judy Beeler, and Kathleen Coelingh, NIAID; no patent; discovery first published in a scientific journal in 1989.

Certiva (North American Vaccine, Inc.) —a combined diphtheria, tetanus, and acellular pertussis vaccine for use in infants and children. A special process that reduces local and systemic adverse events commonly associated with traditional whole-cell DPT vaccine administration has detoxified the acellular pertussis component of this vaccine. Certiva is the first pediatric vaccine introduced into the U.S. market by a new independent vaccine producer in more than 10 years. (Exclusive Patent License Agreement)
NIH Inventors: Ronald Sekura,Yan-Ling Zhang, and Joseph Shiloach, NICHD; first patent application filed in 1986.

Vitravene (Isis Pharmaceuticals, Inc.)— a phosphorothioate oligonucleotide that inhibits cytomegalovirus infections in the eye. Such infections more commonly occur in immunocompromised patients with resultant damage to the retina. Vitravene is the first antisense therapeutic approved for use in humans. (Nonexclusive Patent License Agreement)
NIH Inventors: Jack Cohen, Gerald Zon, Lenoard Neckers, Cy Stein, Shee Loke, Kazuo Shinozuka, and Makoto Masukura, NCI; first patent application filed in 1987.

RotaShield (Wyeth Laboratories, Inc.) —a live oral vaccine for the prevention of rotavirus gastroenteritis in infants. Rotavirus is the single most important cause of epidemic severe acute gastroenteritis (diarrhea and vomiting) in infants and young children in both developed and developing countries. RotaShield is the first rotavirus vaccine approved for use in humans. (Exclusive Patent License Agreements)
NIH Inventors: Albert Kapikian, Harry Greenberg, Richard Wyatt, Robert Chanock, Karen Midthun, Jorge Flores, Yasutaka Hoshino, and Anthony Kalica, NIAID; first patent application filed in 1983.

AcuTect (Diatide, Inc.)—a synthetic peptide radiopharmaceutical used for the detection of acute deep venous thrombosis (DVT). DVT affects an estimated 5 million individuals in the United States each year and is the most common source of pulmonary embolism. AcuTect is the first in vivo imaging agent to target acute DVT in the lower extremities. (Exclusive Patent License Agreement)
NIH Inventors: Frank Robey, Raymond Fields, and Wolfgang Lindner, NIDCR; patent application filed in 1988.

Thyrogen (Genzyme Corporation)—a recombinant form of human thyroid-stimulating hormone for use in follow-up screening of patients who have been treated for thyroid cancer. Thyrogen permits these patients to avoid the debilitating effects of thyroid hormone withdrawal while undergoing standard diagnostic procedures, such as serum thyroglobulin testing and radioiodine imaging. (Exclusive Patent License Agreement)
NIH Inventors: Bruce Weintraub and Fredric Wondisford, NIDDK; first patent application filed in 1989. ■

"'98 WAS A VERY GOOD YEAR."
—Steve Ferguson

Surrogate Endpoints

Biomarkers and Surrogate Endpoints: Advancing Clinical Research and Applications," an international conference cosponsored by NIH and FDA, will be held at the NIH Natcher Conference Center April 15–16, 1999.

NIH Director Harold Varmus, FDA Commissioner Jane Henney, and John Niblack, of Pfizer, Inc., will keynote. For more info, click onto **<http://www4.od.nih.gov/biomarkers>** or contact Saundra Bromberg, 11900 Parklawn Drive, Suite 350, Rockville, MD 20852-2624; (301) 468-6004, ext. 406; e-mail: **<surrogate_endpoints@mdcapconcorp.com>**

Drug Development

In concert with the American Association of Pharmaceutical Scientists (AAPS), NIGMS, NCI, and NIDDK are cosponsoring a meeting, "Membrane Transporters and Drug Therapy," April 8–10, 1999, in Masur Auditorium. Structural, physiologic, genetic/genomic, and pharmaceutical aspects of membrane transporters will be discussed, as well as the role of these proteins in drug discovery, development, and therapy. The meeting is free to all NIH employees. Registration info and agenda can be found at **<http://www.aaps.org/edumeet/nih/index.html>** ■

The MRS Is Calling. . .

The Mitochondrion Research Society (MRS) has been formed to foster interdisciplinary collaborations to advance understanding of mitochondrial biology and the role of mitochondria in such areas as aging, cancer, toxicology, and neurobiology. MRS was founded by Steve Zullo, NIMH, coordinator of the Mitochondria Interest Group (MIG) at NIH (**<http://www-lecb.ncifcrf.gov/~zullo/migDB/>**) and Keshav Singh (**<singhke@jhmi.edu>**), of the Johns Hopkins Oncology Center (JHOC). Membership is currently free. To join, send your name, address, phone number, e-mail address, and research interests to: Cindy Morin, JHOC, 600 North Wolfe Street/Room No. 2-121, Baltimore, MD, 21287, U.S.A.; fax: 410-955-8780. Info can also be found at **<http://www-lecb.ncifcrf.gov/~zullo/migDB/MRS.html>**. ■

Creating a Gene Therapy For Chronic pain and Spinal Cord Disorders

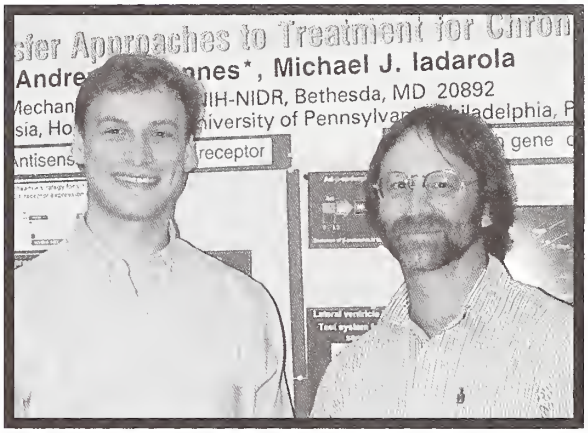
by Michael J. Iadarola, Ph.D., Chief, Neuronal Gene Expression Unit, NIDCR

This research demonstrates a new treatment strategy for chronic pain. It is currently in transition from the lab bench to the patient bedside, as we prepare for a first clinical trial in human subjects. What follows is a personal account of how the research evolved and where it can go in the future. The "paracrine paradigm" we developed is applicable in a general fashion to therapy for chronic neurological disorders.

Pain: Study It or Treat It?

This work began in the summer of 1993 with a small program in therapeutics—small because I was able to carve out only limited time over two summers with an HHMI high school student, Susan Lee (who has since gone on to Harvard Medical School in Boston). I had always been a basic bench scientist, and my lab had been studying synaptic-induced gene regulation in the spinal cord in models of persistent peripheral inflammation. I had discovered that persistent pain up-regulates the opioid peptide dynorphin in the dorsal spinal cord, the first synaptic processing station for pain—first observing this with a radioimmunoassay for dynorphin peptide and later measuring the corresponding mRNA increases, performing studies to localize the spinal neurons involved, and eventually examining seven base pairs of enhancer sequence in the promoter.

The transition to translational research was sparked through our weekly laboratory meetings. What was then the Neurobiology and Anesthesiology Branch contained both basic and clinical research groups, and the clinical group sometimes presented patients with chronic pain problems. This was my first exposure to patients with chronic neuropathic pain disorders, and it was a real eye-opener. Chronic neuropathic pain is notoriously difficult to control with currently available drugs and procedures, and the subjects we were seeing exemplified this clinical state of the art. Often, what had begun as relatively minor nerve damage after a traumatic injury progressed to a severe chronic pain disorder. Patients experienced high levels of spontaneous pain and mechanical allodynia (pain from a normally nonpainful stimulus). Just brushing the skin in the neuropathic zone was enough to cause them excruciating pain. This exposure stimulated us to begin exploring new treatments for pain, in addition to studying the molecular neurobiology of pain.



Fran Pollner

Alan Finegold (left) and Michael Iadarola

First Steps

In choosing among treatment approaches, we wanted to do something new and to use some of the molecular methods that we had expertise in and control over within our own lab. At the time, we were performing transient transfections to investigate those seven base pairs in the dynorphin promoter. Moreover, there was real excitement over the beginnings of gene therapy, much of which was occurring here on the NIH campus. So the idea of adapting techniques of gene transfer to pain treatment seemed like a natural extension of our current program. Still uncertain as to the exact gene to use in pain treatment, we nonetheless needed to assess the basic process of in vivo gene transfer.

That first summer, we asked whether plasmids could transfect neurons or glia in the spinal cord in vivo or in primary cultures. Plasmids were sweeping the literature, and reports had appeared suggesting simple systemic injections were effective at transducing cells. Plasmids were certainly convenient, although I had my doubts about how effective they would be in nonmitotic cells of the nervous system.

The primary cultures worked up to a point: The lacZ test gene expressed β-galactosidase only in the "feeder layer" of flattened glial cells at the bottom of the plate. The neurons, which in these cultures are like groups of round balls sitting atop the flat glia, never seemed to pick up and express the plasmid. In vivo, plasmid transfer was weak, and the

Pain, CNS Disorders Target of Novel Gene Therapy Pathway

continued from page 1

Neuronal Gene Expression Unit in the NIDCR Pain and Neurosensory Mechanisms Branch. "If you inject a virus into brain tissue, it doesn't percolate well through the limited extracellular space in between neurons—and the axonal tracts form a physical barrier against the virus in three-dimensional space—but injection into the CSF will transduce pia mater cells over a broad area," he said. Both the poster and a paper to be published in the May 1 issue of *Human Gene Therapy* were coauthored by Alan Finegold, previously an NIDCR staff fellow and now in the private sector, and UPenn anesthesiologist Andrew Mannes.

It is possible, Iadarola said, that within a year, this "paracrine paradigm" will be tested in humans, in a collaborative NIDCR-NIAMS clinical trial to alleviate pain in terminal cancer patients and, later, in patients with certain inflammatory joint problems—without the side effects associated with opiates.

"Injecting genes into nonneuronal cells to bathe the neurons can work for motor as well as sensory neurons," Iadarola added. "Assuming the disease can be treated with a secreted gene product, like a protein or a growth factor or a neuropeptide, you can go the pial-meningeal route or even into the ependymal cells that line the ventricles or the synovial cells that line the joints. It is not a direct correction of a genetic defect, but it can provide a paradigm" for Parkinson's, multiple sclerosis, spinal cord injury, and many other central nervous system disorders, he said. It may be especially effective, he added, where large sectors of the brain are involved, with the viral vector reaching into the deep recesses of the sulcal spaces after installation into the subarachnoid CSF over the cortex. ■

amount of measurable transgene expression was dismal. We could assay a small increase in β-galactosidase activity biochemically but could not see the cells with histochemical methods. We tried direct injections of plasmid into tissue and even prolonged infusions (for a week, using an osmotic minipump) of about 10 billion plasmid molecules into the cerebrospinal fluid (CSF) space surrounding the spinal cord. We played around with ligand-derivatized polylysines and the timing of plasmid incubations, among other tacks.

Nothing worked very well either in vivo or in the primary culture test system, where the glial cells acted as incredible sponges for DNA, no matter what form it was in or what coating was around it. We needed to look elsewhere.

NOTHING WORKED VERY WELL, EITHER IN VIVO OR IN THE PRIMARY CULTURE SYSTEM, WHERE THE GLIAL CELLS ACTED AS INCREDIBLE SPONGES FOR DNA, NO MATTER WHAT FORM IT WAS IN OR WHAT COATING WAS AROUND IT. WE NEEDED TO LOOK ELSEWHERE.

Virus to the Rescue

We turned our attention to virus. Fortunately, my institute had already established a program in gene therapy run by Brian O'Connell, who helped us get started by providing virus reagents and guidance in filing the necessary paperwork with the Biosafety Committee. We were also lucky to have anesthesiologist Drew Mannes join the group, through a joint agreement with the University of Pennsylvania Anesthesiology Department in Philadelphia. For Mannes, the highest priority for research was that it have clinical relevance.

We began with a straightforward comparison in rats of viral transduction after infusions into the intrathecal space (the CSF space around the spinal cord) or infusions directly into the cord tissue itself (intraparenchymal)[1]. Adenovirus was a vast improvement over plasmid: We achieved nearly 60-fold increases over baseline in β-galactosidase activity upon intraparenchymal injections. We injected directly into the ventral horn to provide a good seal around the cannula tract—and were nearly instantly gratified by transduction of the motor neurons, which turned blue in a matter of minutes. The motor neurons filled up, from the dendritic tree all the way out to the axons in the ventral roots. I thought we had solved the problem of neuronal gene transfer! At the very least, we had one vector we could use for intraparenchymal injection.

Viral infusion into the CSF, however, was not so happy—there was almost no expression in the spinal cord tissue. We found that the pia mater, one of the meningeal layers surrounding the spinal cord, was a very effective barrier to viral entry from the CSF space into the cord tissue proper. We could literally strip off the pia and stain it histochemically for β-galactosidase activity. The pial covering turned blue, but the spinal cord just underneath was devoid of reaction product.

Evolution of the Paracrine Paradigm

At first we reasoned that we would have to break through the pia for virus to gain access to the spinal cord. There ensued a series of increasingly invasive manipulations, starting with hyperosmotic mannitol shock and proceeding to partial enzymatic digestion with intrathecally applied proteases.

None of these strategies succeeded; no virus made it through the pia. We concluded that rather than fight Mother Nature we would attempt to use the pia as a secretory engine. We hypothesized that we could block pain by inducing the pia to secrete a virally transfected analgesic gene product. This is a "gain of function" gene therapy approach.

As luck would have it, we found an ideal gene cassette in the literature. Earlier studies had explored cell transplantation therapy as a potential means of treating pain—using either human cadaver or bovine xenografts of adrenal chromaffin cells (a rich source of enkephalin opioid peptides) or cells that had been engineered to secrete enkephalin. In the latter case, Rusty Gage's group at the Salk Institute in La Jolla, California, had constructed a cassette that allowed fibroblasts to secrete the powerful endogenous opioid β-endorphin. They had fused human β-endorphin at the COOH-terminus of the leader sequence of nerve growth factor (NGF) to direct the secretion of β-endorphin to the nonvesicular secretory pathway.

The idea was to stably incorporate the NGF–β-endorphin cassette into fibroblasts through a retroviral transduction, isolate secreting fibroblast clones, expand the cells, and transplant them into the intrathecal space—a somatic cell gene therapy approach. Gage's group had already characterized the ability of the construct to secrete authentic β-endorphin from cultured fibroblasts but had not used the system in vivo before they dropped this line of research. While the somatic cell–fibroblast approach seemed cumbersome, the cassette itself seemed tailor-made for the connective tissue cells of the pia.

Thus, we were able to simplify the procedure considerably by using direct gene transfer. Fortunately, Gage was able to dig the plasmid out of the freezer and send it to us for subcloning into adenovirus. At this time, Mannes' NIH fellowship ended, and a new postdoctoral fellow, Alan Finegold, joined the group and began making several adenovirus shuttle vectors containing the NGF–β-endorphin cassette and several other sense and antisense constructs.

Here again, the interactive network that characterizes NIH so well provided a helping hand. O'Connell had obtained a contract to produce adenovirus and generously provided us with access to the service, so we obtained several production runs of various viruses.

After some on-the-job training in animal surgery and behavioral research, Finegold was routinely injecting virus into spinal cord and evaluating in vivo transfer. Initially, we directed our injections into the dorsal horn of the spinal cord, where somatosensory signals (such as heat, cold, light, touch, and vibration) are processed. Previously, Mannes had succeeded in transferring genes in vivo into ventral horn motor neurons, but we needed to reach the dorsal horn to control pain.

This proved to be a difficult job for adenovirus, and we slowly came to the conclusion that through no fault in technique, only motor neurons were appropriate targets for adenovirus; the others were apparently impervious to it. Furthermore, we observed that the spread of the virus in the cord was nonuniform. It is an underappreciated fact that the nervous system contains many barriers to free diffusion or dispersal of large viral particles (~90 nM for adenovirus). When the virus

encounters axon bundles, it tends to track along the bundle rather than diffuse through it. Tightly packed cells are another barrier. Aside from these physical issues, the cord appears to unevenly express the receptors for adenovirus binding or attachment (CAR) and internalization (integrins). (We are now investigating receptor sites and targeting strategies.)

Preclinical Testing in Vivo

In the meantime, the viral stocks of the β–endorphin-secreting virus were delivered. We sent some to Mannes in Philadelphia. He infected cells and reported back that the media contained very high concentrations of β-endorphin! Finegold began investigating this in vivo. First, we injected the virus into the lateral ventricle in the brain. This was convenient to examine because CSF could be withdrawn readily from the cisterna magna, which is spatially remote from the ventricular injection site. Andrea Mastrangli's group in the NHLBI Pulmonary Branch had shown that α_1-antitrypsin could be secreted by an adenovirus injected intraventricularly and that the virus entered the ependymal cells lining the ventricle but did not enter the brain tissue proper[2]. This is exactly what we observed as well with co-injection of the β-endorphin–secreting virus and a β-galactosidase–expressing virus. Significant β-endorphin secretion could be measured within 24 hours and reached concentrations more than 10-fold greater than the basal peptide content.

In the spring of 1998, Finegold began rat studies involving intrathecal injections of the virus in conjunction with the application of hot radiant thermal stimuli to the hindpaw. In this test, the rat is unrestrained and can terminate the trial at any time by twitching the paw away from the heat source—a radiant heat lamp with an attached timer. Interestingly, Sprague Dawley rats never cue to the light coming on or the warming phase of the stimulus. However, once the temperature becomes hot, the rat flicks its paw away, which automatically stops the clock and terminates the power to the lamp. Thus, we can obtain an objective measure of nociceptive sensitivity in an unrestrained rat by recording the latency for paw withdrawal. In addition, we can perturb the system by making it hyper-responsive, using an inflammation in one hind paw. Because the inputs to the cord are lateralized, one paw can be used to assess hyperalgesic responses and the other paw of the same animal can be used to assess normal nociceptive responses. Recently, we have used this test to discriminate between different types of pain-reducing drugs. Rob Caudle in our lab has shown that some drugs such as a μ-opiate–selective ligand are analgesic and increase the withdrawal latency in both the inflamed and noninflamed paw. Rob has shown that other compounds have a "pain state–dependent effect," increasing the latency of the inflamed paw only. Certain types of K-opioid agonists (K_2 agonists) and blockers of the *N*-methyl D-aspartate glutamate receptor exhibit this property, which we term antihyperalgesic. Several days after the intrathecal injection of the β-endorphin–secreting adenovirus, we produced a unilateral inflammation and tested the rat's thermal nociceptive responses.

The virus produced an antihyperalgesic effect when the inflamed paw was tested but no effect when the noninflamed paw was examined. Injections of a β-galactosidase–expressing adenovirus did not influence the inflammation-induced hyperalgesia.

In the summer of 1998, we were joined by two students, Jamie Bourque, from the University of Virginia in Charlottesville, and Brian Schulman, an HHMI summer student from the University of Pennsylvania. These two individuals pushed the behavioral aspects of the project to conclusion. They, too, demonstrated the basic antihyperalgesic action of the β-endorphin–expressing virus. They also demonstrated the reversal of this effect by the broad-spectrum opioid antagonist naloxone, indicating that the effect was opioid mediated. These results are in press[3].

We are now using different viruses to increase the longevity of expression and designing cassettes for regulated control. We hope to be testing the adenovirus in chronic pain patients in the near future. Exactly when will depend on how the toxicology results turn out.

Implications and Further Steps

Our studies have delineated a direct in vivo approach for treating pain using gene therapy techniques. Finegold coined the term "paracrine paradigm," because the therapeutic gene product is secreted by cells in the vicinity of the relevant neurons. This approach represents a new way to deliver peptides to the nervous system. One can imagine a host of new avenues to peptide pharmacology when a "genetic generator" for peptide production is deposited in or near the target tissue. One of the strengths of this approach, therefore, is its versatility—all 20 amino acids are at one's command. It also bypasses one of the major stumbling blocks to using peptides as drugs—delivery. Working with the spinal cord makes the paracrine approach easy. The spinal subarachnoid CSF space is readily accessible by lumbar puncture, a common medical procedure, and injections by lumbar puncture may eventually suffice to place the viral vector into the pia. Brain disorders such as Alzheimer's disease that affect many gyri could be approached in a similar fashion. Multiple injections of some "rescue gene" directly into brain tissue would be somewhat invasive, but infusion of a viral vector into the subarachnoid space may distribute the therapeutic vector very effectively. We are interested in exploring this possibility in a larger animal, such as a monkey, but not in a rat because the rat is lissencephalic (has a smooth brain with no gyri and sulci). But some advantages also impose some constraints. At the moment, we are using gene products that act extracellularly, on a cell surface receptor or a transmitter. The paracrine approach cannot directly correct a defective neuronal gene, nor is it likely to supply a critical intracellular protein. But any condition that could benefit from exposure to growth factors, such as spinal cord trauma, would be a prime candidate for this approach. This is an area we hope to address in future collaborative studies. The incredible simplicity and relative noninvasiveness of the paracrine approach provides a new frame of reference for in vivo gene therapy of the nervous system. ■

THIS APPROACH REPRESENTS A NEW WAY TO DELIVER PEPTIDES TO THE NERVOUS SYSTEM.

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Recently Tenured

Paul Love *received M.D. and Ph.D. degrees from the University of Rochester (N.Y.) in 1987 and completed a residency in clinical pathology at Washington University in St. Louis. He joined NICHD as a medical staff fellow and became head of the Unit on Cellular and Developmental Biology in 1993. He is now a senior investigator in the Laboratory of Mammalian Genes and Development.*

Fran Pollner

Paul Love

My research interests are directed at understanding the cellular and genetic events that regulate T lymphocyte development. A major focus of my work has been on the role in thymocyte maturation of signals transduced by cell surface receptors, particularly the T-cell antigen receptor (TCR). In mature T cells, the TCR is centrally involved in antigen recognition, T-cell activation, and cell-mediated immunity. In developing thymocytes, TCR signals are important for maturation and thymic (positive and negative) selection. How the TCR is capable of orchestrating these various processes remains a central question in immunology.

In a series of experiments conducted over the past several years, my lab has systematically dissected and characterized the function of the multiple signal-transducing sequences contained within the TCR complex. TCR signal-transduction sequences (termed immunoreceptor tyrosine-based activation motifs; ITAMs) are contained within four distinct subunits of the multimeric TCR complex (ζ, CD3-γ, -δ, -ε) and are triplicated in the ζ–chain cytoplasmic domain. Di-tyrosine residues within ITAMs are phosphorylated upon TCR engagement and function to recruit signaling molecules, such as protein tyrosine kinases, to the TCR complex, thereby initiating the T-cell activation cascade. To examine the role of individual TCR signaling subunits in development, we generated knockout mice lacking either ζ or CD3ε by gene targeting. The phenotype of these mice revealed critical functions for these subunits in promoting TCR surface expression and T-cell development. The importance of specific TCR-ITAMs was then examined by reconstituting TCR surface expression in $\zeta^{-/-}$, $CD3\varepsilon^{-/-}$, or $\zeta^{-/-} \times \varepsilon^{-/-}$ mice using transgenes that encode full-length ζ- and/or ε-chains or ITAM mutant (signaling-defective) variants of these proteins. Examination of T-cell development in these mice revealed the striking finding that neither ζ-chain nor CD3ε signals are specifically required for T-cell maturation. Thus, the multiple TCR signaling motifs appear to have redundant signaling functions. We further learned that the multiple ITAM configuration of the TCR is especially important for thymocyte selection (a developmental process that ensures maturation of self-educated T cells and prevents maturation of potentially autoreactive T cells). These results identify an in vivo function for the multiple ITAM-TCR structure in signal amplification.

We have also used these TCR signaling variant models to search for other cell-surface structures that can influence (positively or negatively) the TCR signaling response. The analysis of one such molecule, CD5, which has been shown to negatively regulate TCR signaling and to participate in thymocyte selection, constitutes another area of investigation in the laboratory. We found that CD5 surface levels are developmentally regulated by TCR signaling intensity and by the affinity of the TCR for selecting ligands. These results support a role for CD5 in modulating or fine tuning the TCR signaling response during development.

Finally, my lab is searching for genes that may be important for thymocyte maturation. These experiments have led to the identification of a novel protein tyrosine kinase (Txk) and protein tyrosine phosphatase (PTPK1). The phenotype of Txk transgenic mice generated in our lab indicates that Txk functions in the pathway leading to calcium mobilization after TCR engagement.

We are generating PTPK1-deficient mice to examine the role in lymphocyte development of the protein tyrosine phosphatase PTPK1, which is expressed in immature hematopoietic stem cells and early thymocytes. Similar strategies are currently being used to identify other genes in the early fetal thymus that may be important for T-cell development or T-lineage commitment. Once identified, transgenic and/or knockout (null) mutations of these genes can be generated to study their function in lymphopoiesis. ■

Smoke Signals

The signs are now posted all over campus: "Smoke-Free Area" signs extend to certain outdoor areas what has been a standing prohibition against using lighted tobacco products inside NIH buildings. These outdoor areas include all building entrances and exits, air-intake ducts, loading docks, covered parking garages, and designated courtyards.

The prohibition is in accordance with an executive order issued in 1997 mandating that all federal agencies protect employees and visitors from the health risks of environmental tobacco smoke. An updated NIH Smoking Policy, crafted by an NIH committee composed of smoking and nonsmoking employees and signed by NIH Director Harold Varmus last May, can be found on the web at **<http://www1.od.nih.gov/ohrm/qwl/smokepol.htm>**.

The policy applies to all NIH employees, other federal employees, and members of the public who are working in or visiting facilities owned, leased, or controlled by NIH.

Further, NIH strongly encourages and supports employees who want to break the smoking habit. Anyone interested in smoking cessation programs may contact the NIH Employee Assistance Program at (301) 496-3164. ■

Let's Go, CAPS!!!

Small laboratory animals used by NIH intramural scientists will soon be available for online purchase.

Thanks to the combined efforts of the Veterinary Resources Program (VRP), CIT, and NIMH—and numerous consultants from NIH Intramural Animal Programs—VRP is piloting the Central Animal Procurement System, or CAPS.

CAPS will replace the current cumbersome paper process and be accessible via PCs or DELPRO terminals. Linked to the ADB and Central Accounting System, it will automatically bill ICs and generate data to enable prompt payment of vendors. The system has been designed with built-in levels of authority for investigators, IC (animal-procurement) approving officials, animal-facility managers, IC animal-facility receiving technicians, animal-program directors, VRP animal-procurement staff, the Office of Financial Management/Accounts Payable, and the NHLBI Contracts Operations Branch Servicing Center.

As soon as the kinks are worked out in the pilot—with NIMH—the remaining NIH animal programs will be brought online. It's anticipated that CAPS will be fully operational in late spring or early summer 1999. ■

—Pamela Dressell, ORS

New Antibody-Purification Method

NHLBI investigators have developed a new protein-purification method—centrifugal precipitation chromatography—that uses ammonium sulfate and potassium phosphate buffer only (no solid support). They have used the method to purify and concentrate monoclonal antibodies—immunoglobulin M (IgM)—against mast cells and think it may be equally successful in purifying either IgM or IgG from a culture medium or an ascitic fluid.

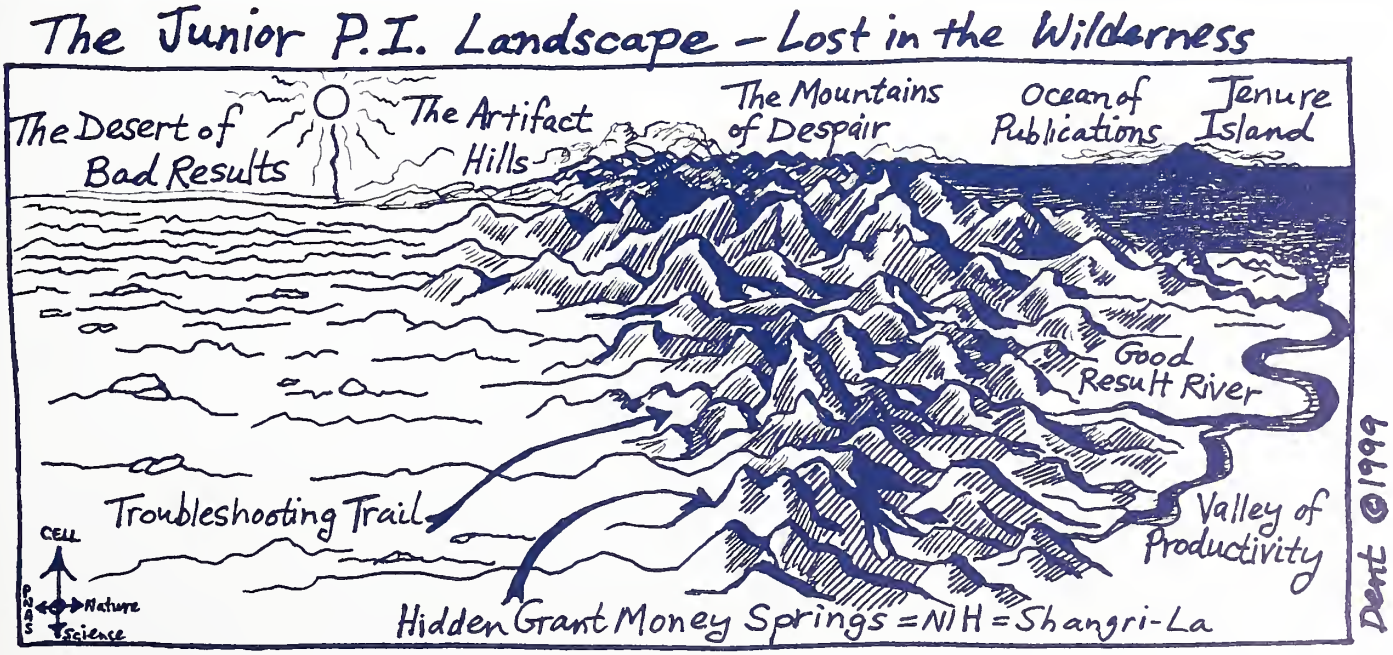
Because they are quite interested in testing the capability of the method in purifying antibodies and other proteins, the NHLBI researchers are putting themselves at the service of other intramural researchers with problems purifying antibodies. Anyone with such a problem should contact Yoichiro Ito, who can be found in Building 10, Room 7N322, and can be reached by phone at 496-1210, by fax at 402-3404, and by e-mail at <**itoy@gwgate.nhlbi.nih.gov**>. ■

VRP Pharmacy On-line

The Veterinary Resources Program Pharmacy, in Building 14A, offers one-stop shopping for veterinary and human over-the-counter or prescription products. Inventory and instructions for ordering are on the web at <**http://dirs.info.nih.gov/intramur/vrp/pharmacy.htm**>. You can also hot-link to this site from the VRP Home Page/Description of Services/Pharmacy at <**http://dirs.info.nih.gov/intramur/vrp/services.htm**>. ■

Biomedical Imaging

The NIH Bioengineering Consortium (BECON) will host its 1999 symposium, "Visualizing the Future of Biology and Medicine," on June 25–26 at the Natcher Conference Center on the NIH campus in Bethesda. Bioimaging advances in disease detection, diagnosis, and therapy are on the agenda. For registration and more information, visit the BECON symposium web site at <**htpp://www.nih.gov/grants/becon/meeting99/index.htm**>. Anyone who would like to present a poster should contact Phil Chen at 496-3561 **immediately**. ■



Call for Catalytic Reactions

In this issue, we are asking for your reactions in four areas: the NIH Academy, an NIH graduate school, mentoring, and *The NIH Catalyst* itself.

Send your responses on these topics or your comments on other intramural research concerns to us via e-mail: <catalyst@nih.gov>; fax:402-4303; or mail: Building 1, Room 209.

In Future Issues...

- Vaccine Research Center
- Space: The Final Frontiers
- Getting IT at NIH

1) What do you see as major objectives for an NIH Academy, and by what means would you achieve them?

2) Do you think there is a need for an NIH graduate school that utilizes NIH expertise in translational and clinical research? Would you like to serve on the faculty?

3) Do you think the emphasis on mentoring at NIH is appropriate? Should there be programs to teach mentoring/training skills?

4) Do you have any suggestions for improving the form or content of *The NIH Catalyst?*

The NIH Catalyst is published bi-monthly for and by the intramural scientists at NIH. Address correspondence to Building 1, Room 209, NIH, Bethesda, MD 20892. Ph: (301) 402-1449; fax: (301) 402-4303; e-mail: <catalyst@nih.gov>

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